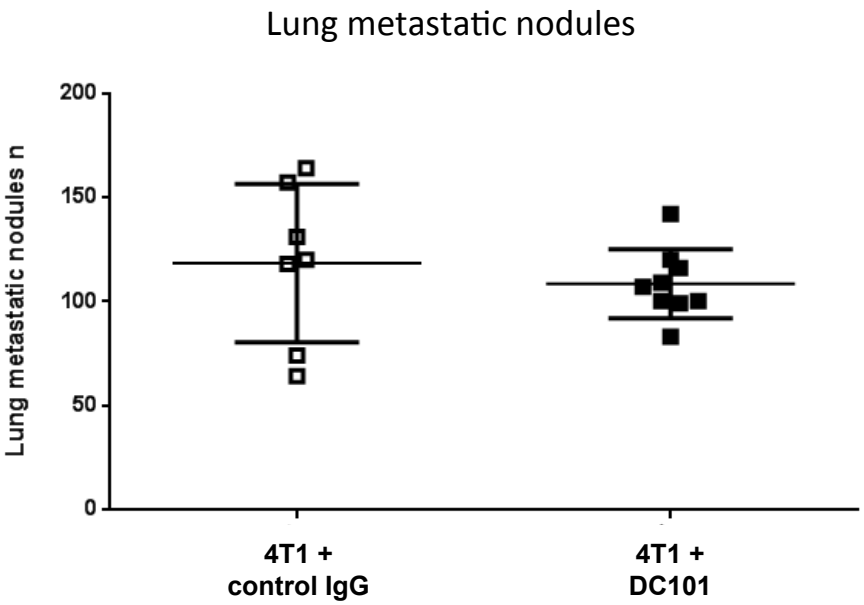

Figure S1. DC101 treatment does not show anti-metastatic activity in an experimental i.v. model of lung metastasis. 4T1 cells were injected in the tail vein and lung metastases let to develop for 9 days. Treatment with DC101 or IgG isotype control was initiated at day 1 post cancer cell inoculation and followed the same schedule as for the orthotopic model. Quantification of lung metastasis nodules in the two treatment groups. Ten mice per group were used.

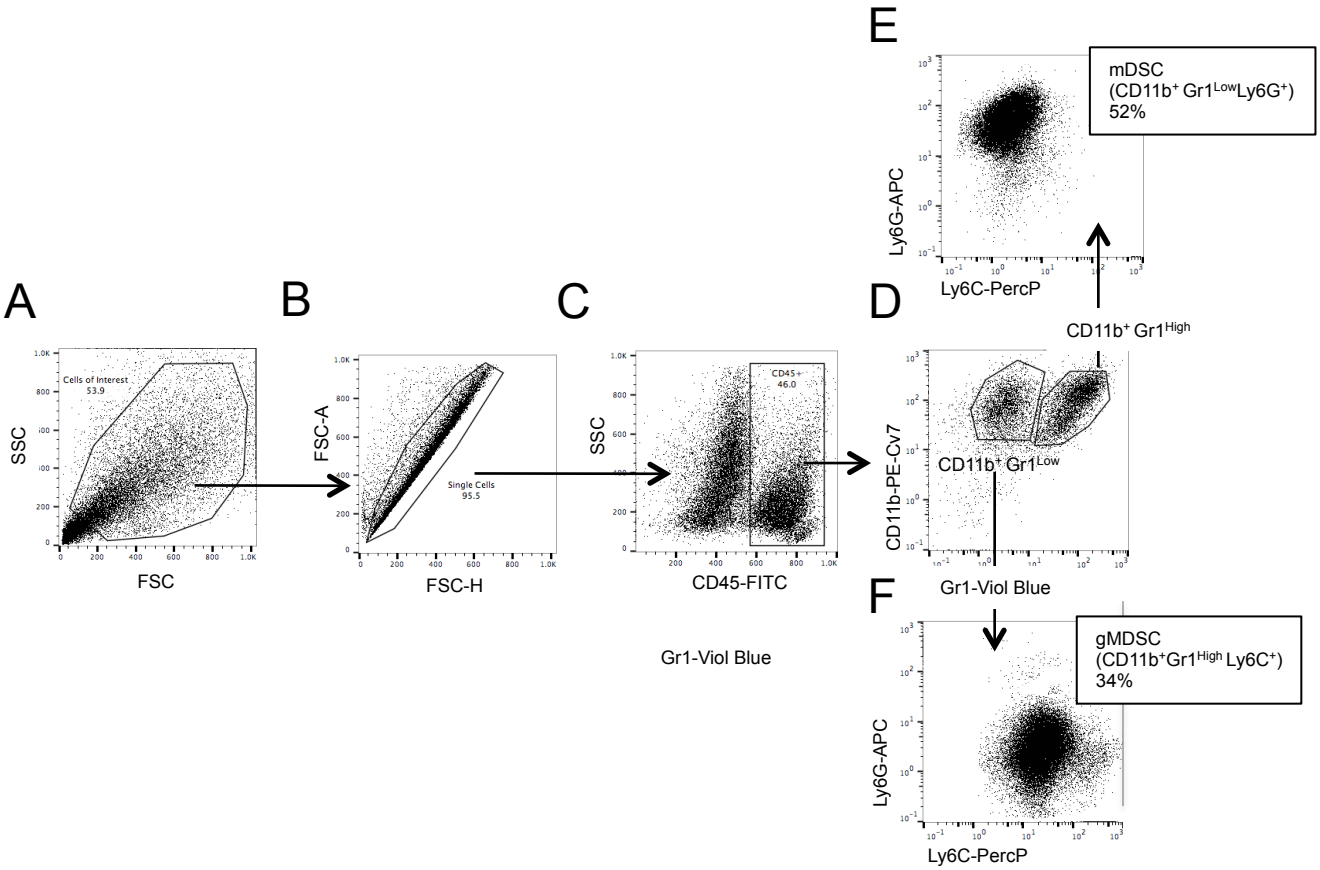

Figure S2. Representative images of flow cytometry dot plots to demonstrate the gating strategy used for the analysis of MDSC in 4T1 tumor bearing mice. A: SSC/FSC gating of lymphocytes/monocytes isolated from tumors. B: FSC-A/FSCH gating to exclude doublets from the cell population of interest. C: $CD45^+$ cells, were gated for further analysis. D: CD11b Gr1 double staining of $CD45^+$ cells. Gated populations indicate $CD11b^+Gr1^{Low}$ and $CD11b^+Gr1^{High}$ cells. E-F: Ly6C and Ly6G double staining of $CD11b^+Gr1^{High}$ and $CD11b^+$ $Gr1^{Low}$ cells respectively define the mDSC ($CD11b^+$ $Gr1^{Low}Ly6C^+$) and gMDSC ($CD11b^+Gr1^{High}$ $Ly6G^+$) cell populations analyzed in this study.

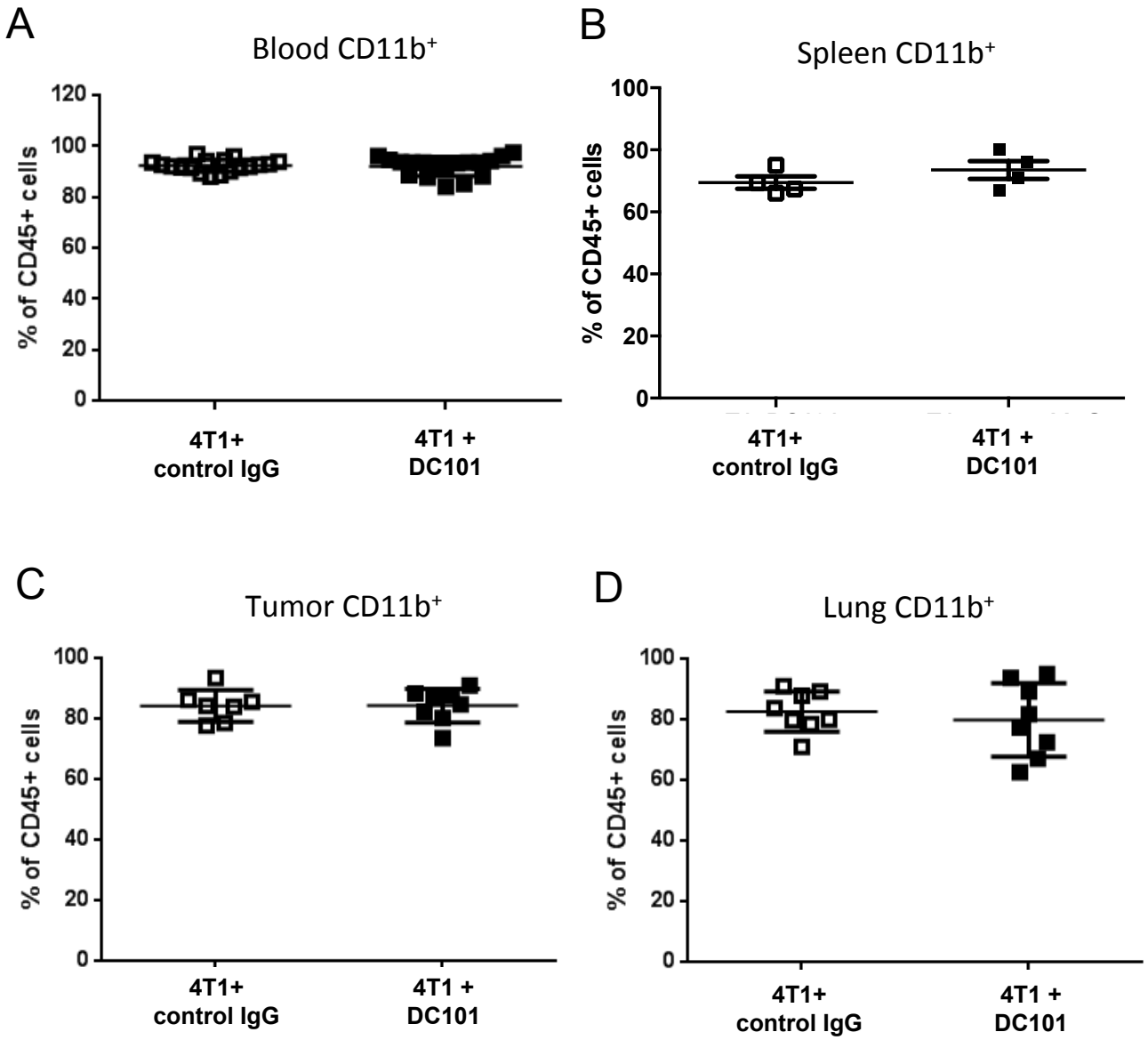

Figure S3. DC101 treatment does not alter the frequency of circulating CD11b+ cells. Frequency of CD11b+ cells in the blood (A, day 22), in the spleens (B), in primary tumors (C) in metastatic lungs (D) of mice treated with DC101 (4T1+DC101) or IgG control (4T1+control IgG) antibodies. N=3, mice analyzed per group=8-20.

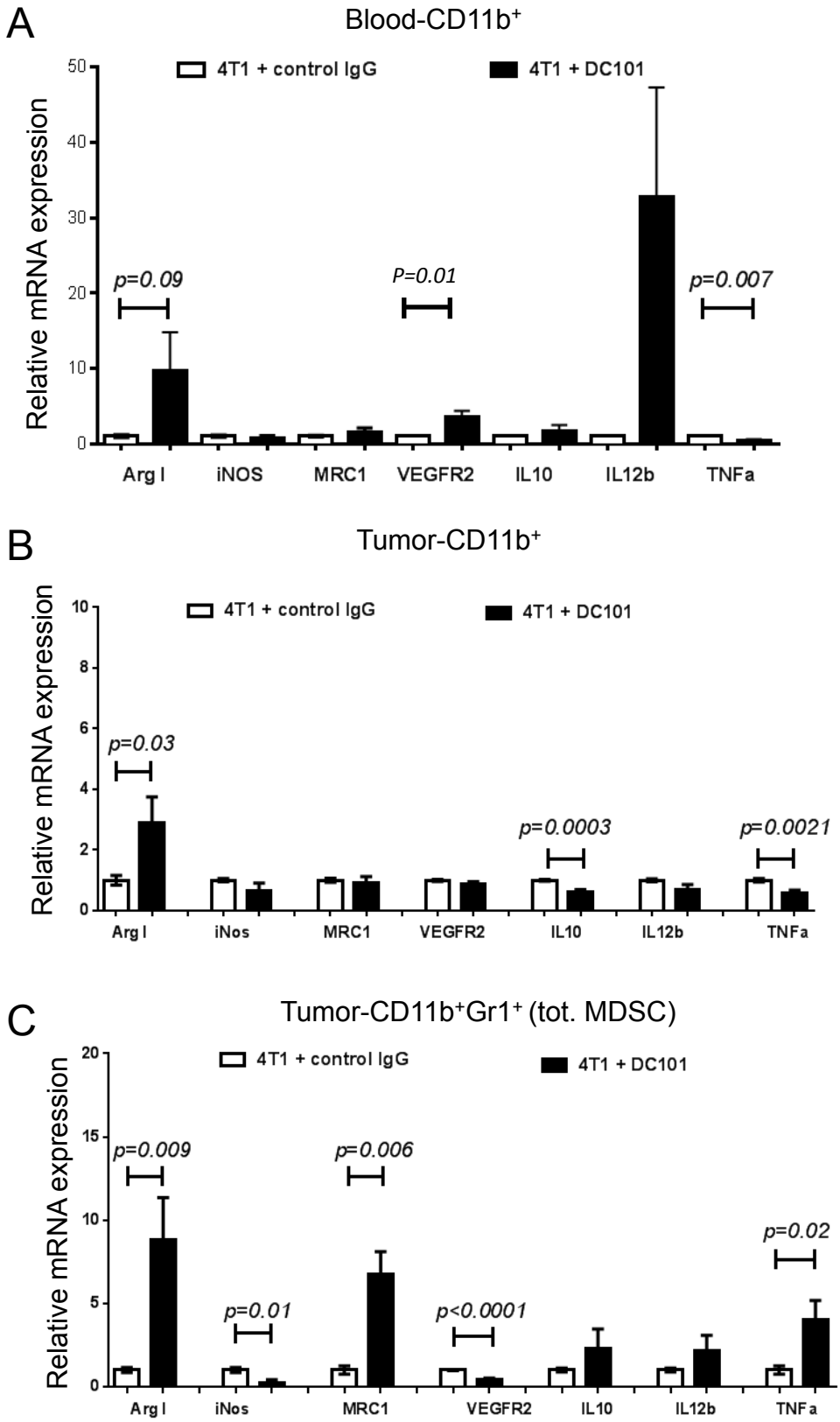

Figure S4. Effect of DC101 treatment on the expression of Arg I, iNOS, MRC1, VEGFR-2, IL10, IL12b, TNFα mRNA expression in CD11b$^+$ and CD11b$^+$Gr1$^+$ cells in blood and tumor. A: Relative mRNA expression level for the indicated genes in blood circulating CD11b$^+$ cells. Mice analyzed per group= 4-5. B: Same analysis for tumor-derived CD11b$^+$ cells. Cells were positively isolated from DC101-treated (4T1+DC101) or IgG control-treated (4T1+control IgG) mice by MACS. Mice analyzed per group= 9-10. C: Same analysis for tumor-derived CD11b$^+$Gr1$^+$ cells. Cells derived from both treatment groups were positively isolated by MACS and subsequently sorted by FACS. mRNA expression was measured by real time RT-PCR. Mice analyzed per group= 4-5. For each gene the value in control treated mice was set equal to 1.

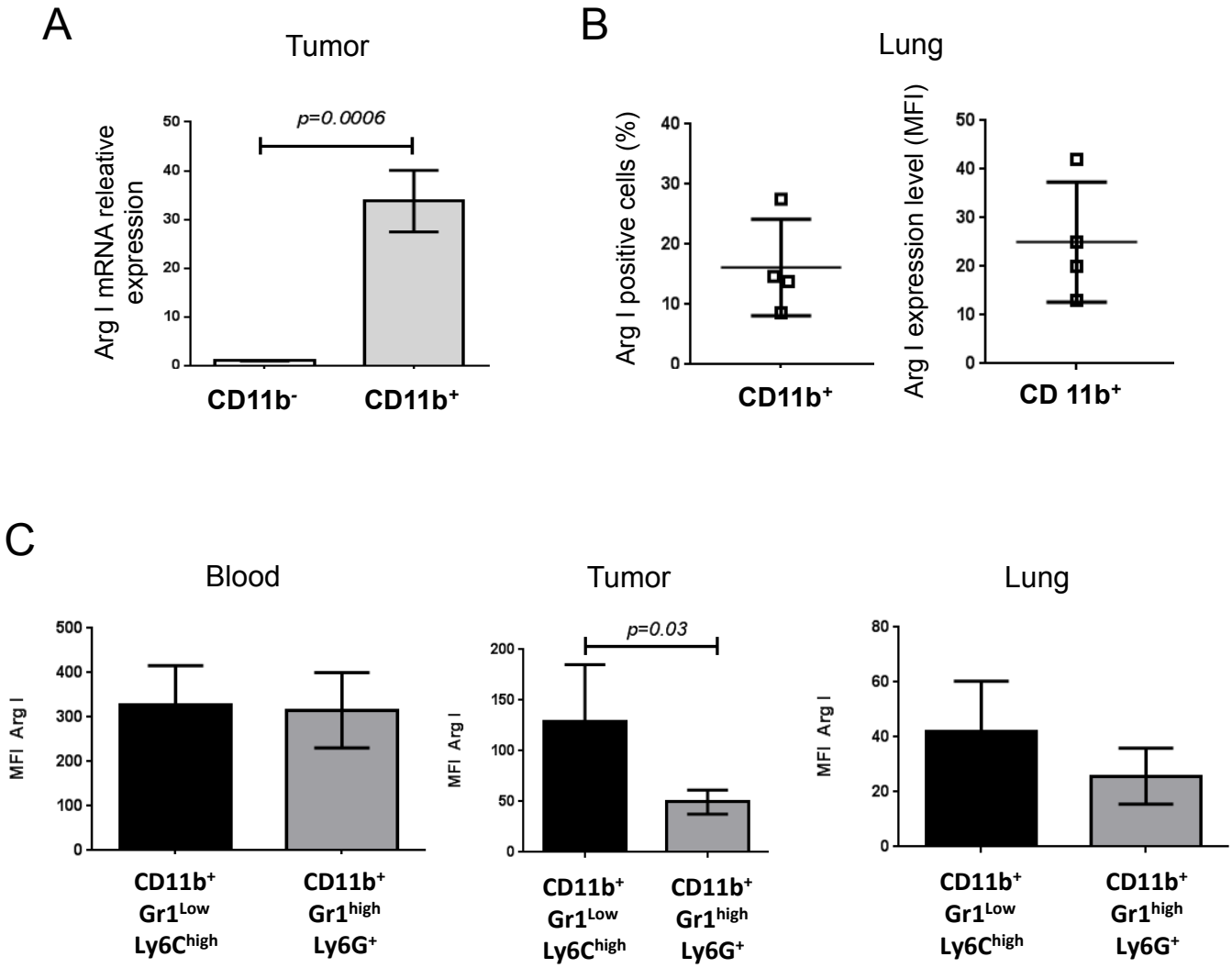

Figure S5. Arg I expression in CD11b$^+$ cells. A: CD11b$^+$ cells are the main source of Arg I in the tumor microenvironment. CD11b$^+$ and CD11b$^-$ cells were MACS sorted from dissociated tumors and analyzed for Arg I mRNA expression by real time RT-PCR. N=2; mice analyzed per group=5. B: Arg I expression analysis by flow cytometry in CD11b$^+$ cells isolated form metastatic lungs expressed in % of positive cells (Left) and mean fluorescence intensity (MFI, right). C: Arg I expression analysis by flow cytometry in CD11b$^+$Gr1^{Low}Ly6C$^+$ and CD11b$^+$Gr1^{High}Ly6G$^+$ cells in the blood, tumor and metastatic lungs. CD11b$^+$Ly6C$^+$ and CD11b$^+$Ly6G$^+$ cells are 100% positive for Arg I expression. Level of expression is highest in blood, intermediate in tumor and low in lung. N=2; mice analyzed per group=4. MFI, mean fluorescence intensity.

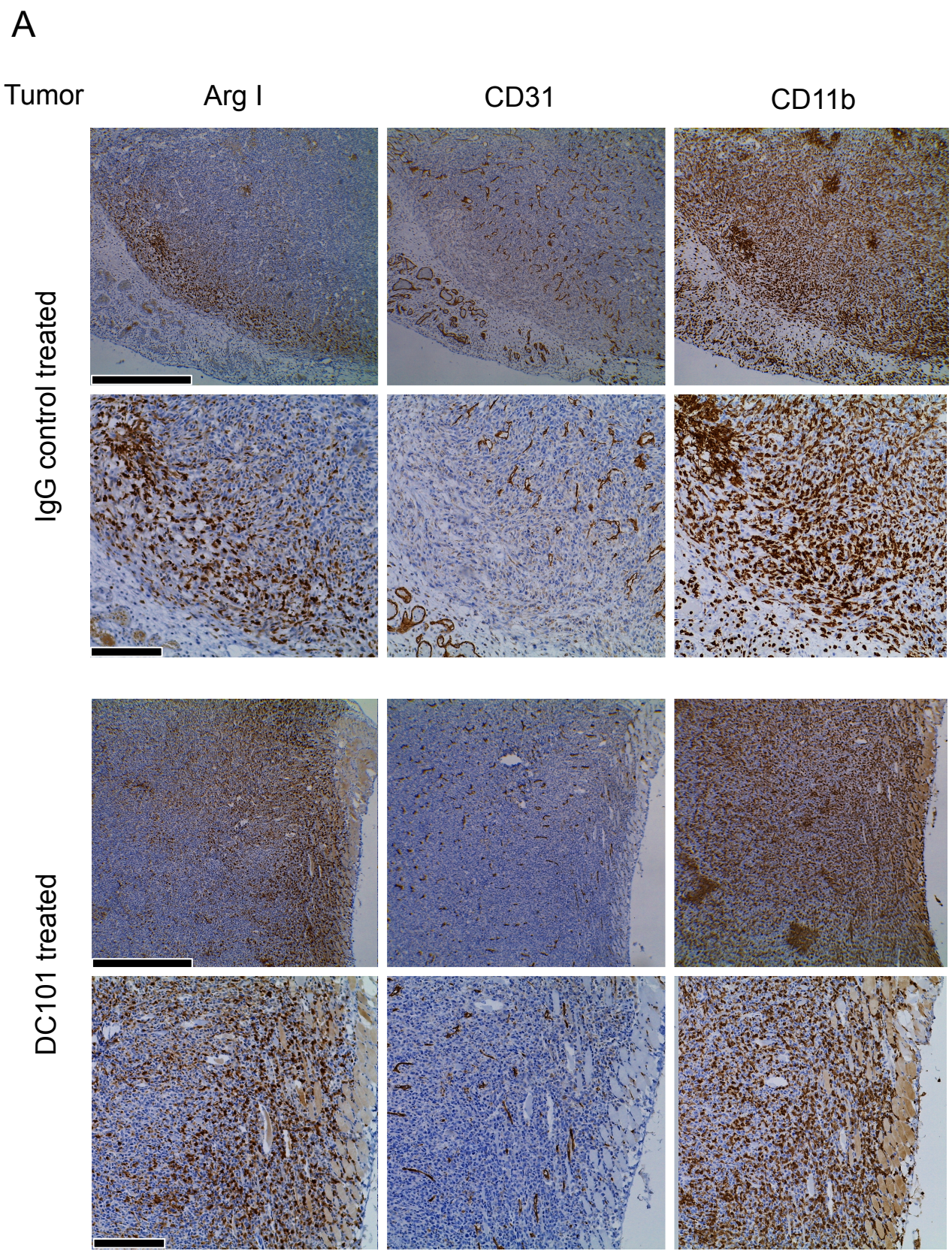

Figure S6

B

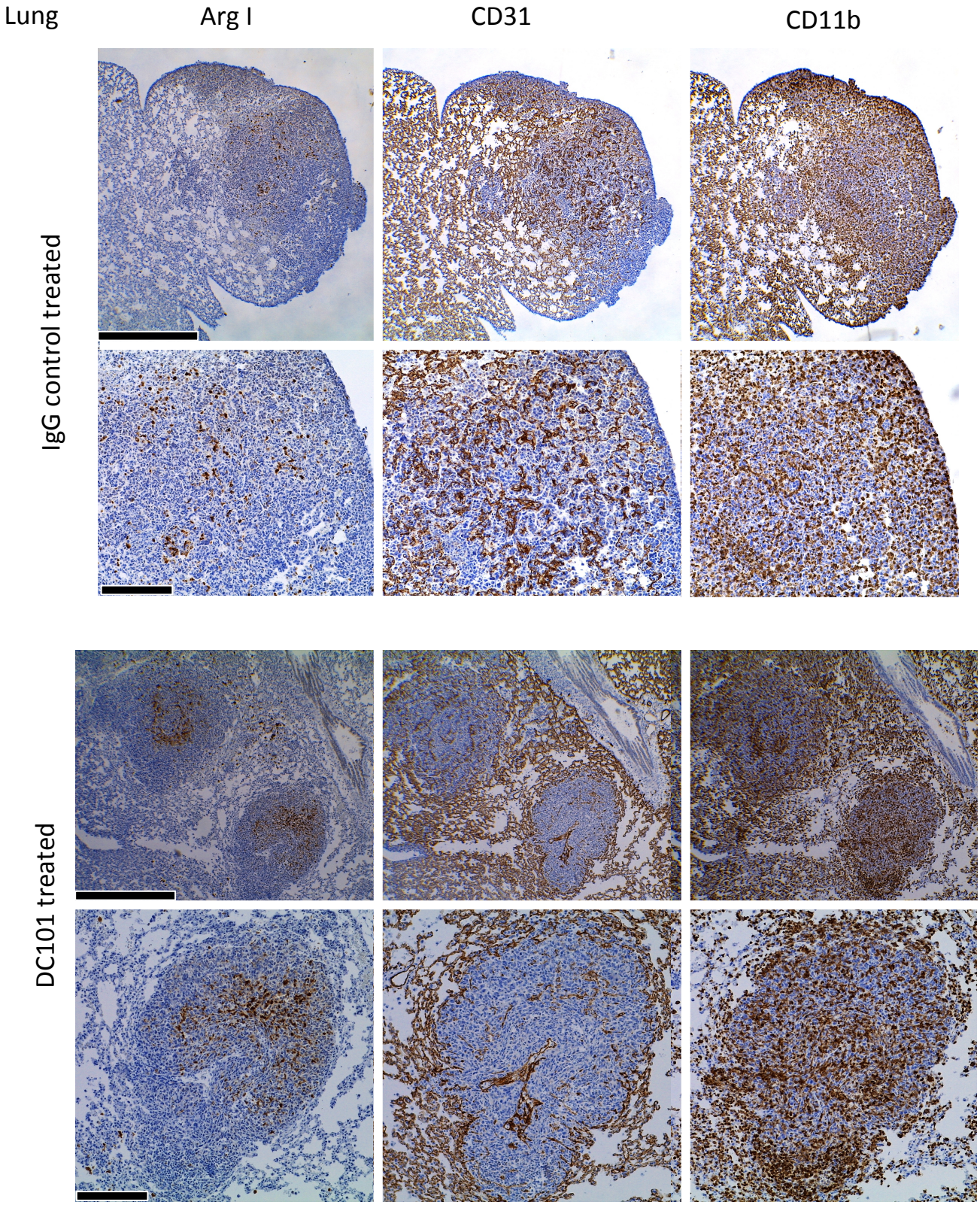

Figure S6. Immunostaining for Arg I, CD31 and CD11. A, B: Tumors (A) and lungs (B) from control IgG and CD101-treated mice were stained with antibodies to Arg I, CD31 and CD11b. DC101 treatment decreases microvascular denisty and increased Arg I positive cells in the lesions. Scale bars: upper raws, 1000 µm; lower raws 250 µm.

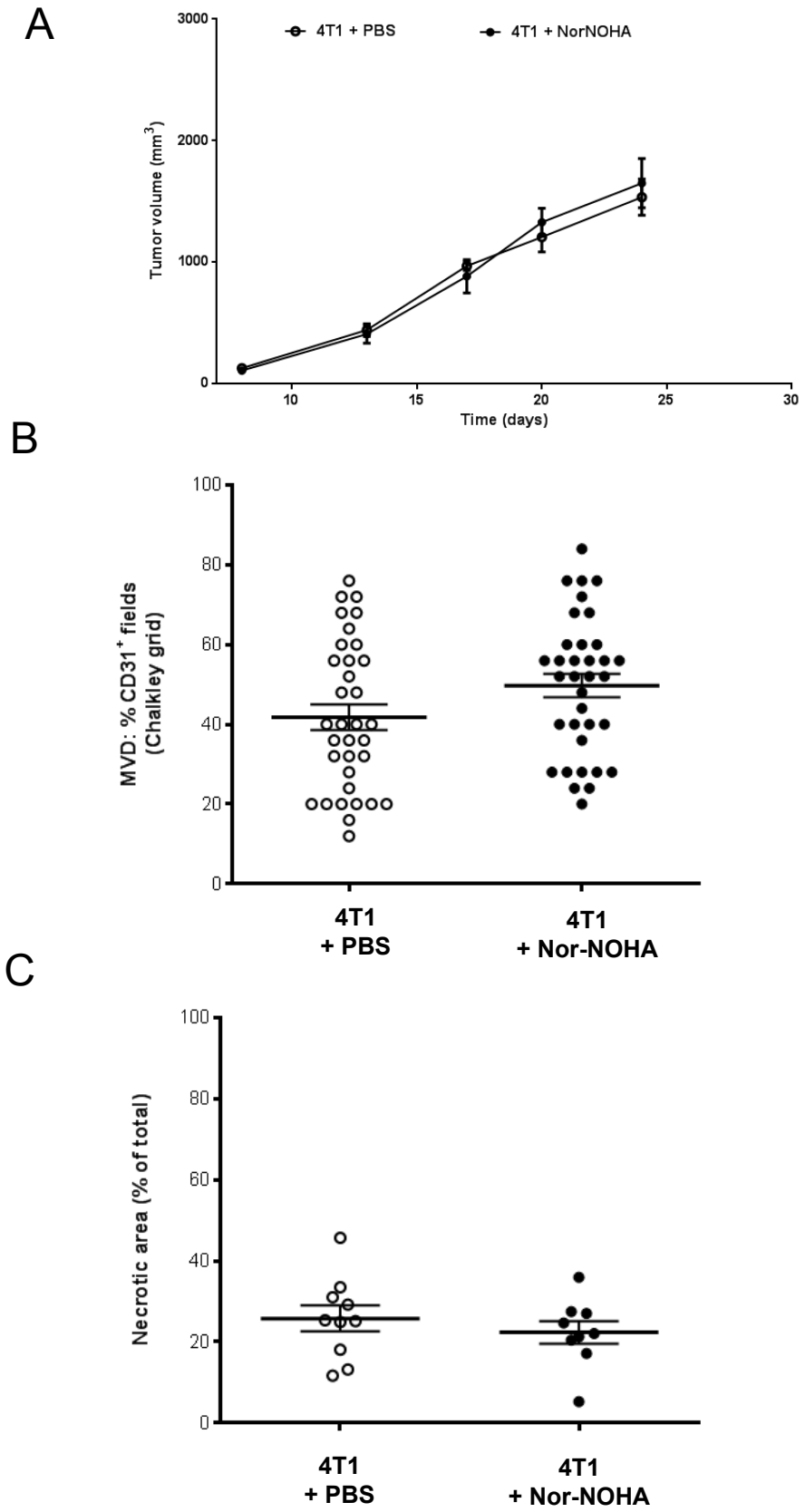

Figure S7. Nor-NOHA treatment does not affect primary tumor growth. A: Growth curves of 4T1 tumors in mice treated with Nor-NOHA or PBS. B: Quantification of tumor microvascular density (MVD) performed on 4-6 representative images derived from both treatment groups. C: Quantification of tumor necrosis by H&E staining and morphometric analysis. Nor-NOHA-treated mice: 4T1+Nor-NOHA; control treated mice: 4T1+PBS. N=1-2, mice analyzed per group = 7-10.

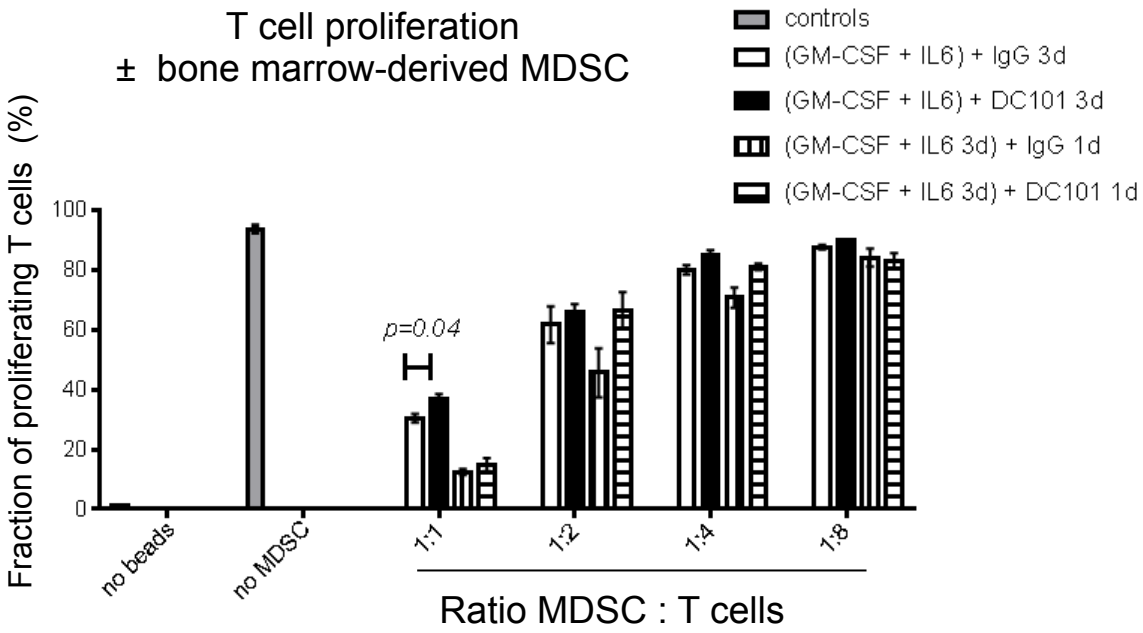

Figure S8. Effect of DC101 on inhibitory activity of *in vitro* generated BM-MDSC. MDSC were generated *in vitro* by exposing bone marrow progenitor cells to GM-CSF and IL6 during 3 days. DC101 or control IgG were added during the 3 days (3d) or at the last day (1d) of culture, as indicated. They were positively isolated by MACS and mixed with stimulated T cells at the indicated ratios. Results from one representative experiment per condition is shown; analysis done in triplicate.